SEROLOGICAL INTERRELATIONSHIPS BETWEEN THE PLAGUE MICROBE AND BACTERIA OF THE INTESTINAL GROUP

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Following is the translation of an article by R. S. Mikhaylova, Scientific-Research Antiplague Institute for Kavkaz and Zakavkaz, published in the Russian-language periodical Trudy Armyanskoy Protivochumnoy Stantsii (Trudy of the Armenian Antiplague Station) No 3, 1964, pages 143--151. Translation performed by Sp/7 Charles T. Ostertag, Jr.

The problem concerning antigenic bonds between bacteria which are related to various genera and families has attracted the attention of many investigators.

G. Schutze (1928) discovered the O-bond between Pseudotuberculosis rodentium and strains of the Salmonella group B. M. Toucas, G. Girard and L. LeMinor (1956) demonstrated the presence of common antigens in P. pseudotuberculosis rodentium type IV and strains of Salmonella group B. In the opinion of Ye. I. Smirnova and Ye. V. Chibrikova (1960) the causative agent of pseudo tuberculosis of rodents possesses an antigenic affinity with bacteria from the Salmonella group, by which it is differentiated from the plague microbe. Based on the data of L. A. Timofeyeva, R. R. Krivolyapina and G. V. Yakubovskaya (1954) and Sokolova (1959), the causative agents of paratyphoid B and typhoid fever may be agglutinated by plague serum in high titers. In tests of precipitation in gel (1960) S. I. Zaplatina established the presence of common antigens in the plague microbe and the causative agents of paratyphoid diseases and dysentery.

It must be pointed out that up until recently a limited number of strains of P. pestis and families of enterobacteria have been compared in the appropriate tests. In connection with this, it would have been difficult to judge on the regularity of an antigenic affinity between the stated microorganisms, is there a connection between the presence in the plague microbe of these or those antigens, common with enteric bacteria, with other biological properties of the cultures, their origin, etc.

The aim of the present investigation is an accumulation of the materials on these problems.

Materials and Methods

346 strains of the plague microbe were subjected to the investigation. These were isolated in 1952--1962 in the area around the Caucasus. Of these, 88 strains were obtained from susliks and their fleas in the Dagestan ASSR, 228 strains -- from red-tailed gerbils and fleas in the territory of Azerbaijan, 8 strains -- in the same territory from other species of rodents (jerboa, social vole). At the time of study all of the above stated strains were found in the typical plague R-form. Based on their properties the overwhelming majority belonged to the suslik variety of P. pestis. Of the cultural peculiarities of the strains, it must be noted that 244 of them fermented lactose, 44 -- fermented rhamnose, 4 -- did not oxidize glycerin, 1 -- broke down urea, 4 -- possessed a nitrifying ability, and 11 -- reduced nitrates to nitrites.

Besides this, the tests included 22 strains of P. pestis, isolated in 1962 in the territory of the Armenian highlands from common voles and their fleas. Distinctive characteristics of these cultures, be ides ecology, were denitrification ability, activity in respect to rhamnose and a sharply lowered pathogenicity for guinea pigs.

All the above enumerated strains were tested in the agglutination reaction with the sera of intestinal bacteria: S. paratyphi A, S. typhi murium, S. suipestifer, S. enteritidis, E. coli 0-111, E. coli 0-55, E. coli 0-26. The reaction was set up on glass by the usual method. The sera were preliminarily diluted with physiological solution 1:25. Positively reacting strains were subsequently checked with the above named sera in the developed agglutination reaction, and also with moncreceptor salmonellosis 0 -- sera I, II, IV, V, VI_2, VII, VIII, IX, III--X.

165 strains of various species of bacteria of the intestinal group, isolated from susliks, gerbils, birds, humans and other objects, were investigated with plague agglutinating serum (titer 1:2560) by the method of the orientating reaction on glass. From the rodents and birds the appropriate cultures of bacteria were obtained by means of seedings from internal organs, contents of the small and large intestine, on selective nutrient media (Ploskirev and Levin agar). The species composition of the intestinal bacteria studied is indicated in the next section of the present report. More detailed characteristics of these cultures are presented in separate reports (Mikhaylova, Gusev, 1960; Mikhaylova, Guseva, Gusev, 1961).

Positively reacting cultures of intestinal bacteria were studied in a detailed agglutination reaction with plague agglutinating serum.

The subsequent study of the antigenic interrelationships between strains of P. pestis and enterobacteria was carried out by menas of setting up the cross agglutination reactions and by the method of antibody

adsorption. For obtaining immune sera to the P. pestis strains, rabbits weighing 2--2.5 kg received intravenously 1 billion microbial bodies in 1 ml of physiological solution. The injections were repeated 3--4 times with weekly intervals. For immunization with avirulent cultures we used a live culture, and we prepared the formalinized antigen from virulent strains. Intestinal O-antisera were obtained by means of immunizing rabbits with cultures of bacteria which had been preliminarily boiled. Immunization was carried out by the method of F. Kaufman (1959).

For the reaction of antibody adsorbtion, cultures of P. pestis were used which had been inactivated with formalin. Inactivation of the cultures of intestinal bacteria was performed by heating at 100°. The exhausting dose was determined depending on the results of a preliminary saturation of the serum with a homologous strain. Precipitation of the microbial suspension was carried out by centrifugation. The adsorbed sera were tested with exhausting and homologous strains in dilutions of 1:50 and up to the limiting titer of the serum.

Results of the Investigation

Out of 346 strains of P. pestis investigated, 31 strains (8.9%) produced a positive agglutination reaction with one or the other sera of intestinal bacteria. It must be noted that 8 strains were agglutinated with the intestinal antisera of two species and 7 strains -- with the sera of three species. As is seen in table 1, a positive result was recorded most often of all with the sera of S. typhi murium, S. enteritidis, and also S. paratyphi C. Only 2 strains of the plague microbe (1:100) reacted with the serum of S. paratyphi A. With the sera of coli-pathogenic bacteria (O-111, O-55, O-26) the positive reactions were observed with almost the same frequency.

In checking the 31 strains of the plague microbe with monoreceptor salmonellosis O-sera, 17 of these reacted with one or several sera to the somatic antigens I, II, IV, VI_2, VIII, IX and III-X.

A comparison of the results of studying the serological interrelationships with other data, characterizing the strains of P. pestis (origin, cultural and biochemical peculiarities, virulence, relationship to specific bacteriophage, etc.), did not expose sufficiently conclusive bonds. During an analysis of the materials, at first the impression was created that the presence of a serological community with intestinal bacteria is inherent primarily to strains of the plague microbe which had been stored for a long time on artificial nutrient media. However, several strains of P. pestis, isolated in the territory of Armenia in 1962, were agglutinated by intestinal antisera already in the first generations.

Out of 165 cultures of bacteria from the intestinal group, 46 strains

(27.8%) reacted with plague serum in titers of 1:50--1:1600 (table 2). Positive reactions were given most often by group "B" salmonella. Thus, out of 21 strains of S. typhi murium, 13 strains were agglutinated by plague serum, including 2 strains in dilutions of 1:1600. In the investigation of 8 strains of S. paratyphi B a positive result was obtained in 5 of them, and in one case it was also in a dilution which is close to the limiting titer. Positive results were obtained with all the remaining investigated species of intestinal bacteria, excluding S. paratyphi A. Attention is drawn to the circumstance that some of the strains of E. coli communis, which were obtained from susliks and gerbils, were agglutinated in high titers.

In the tests of the cross agglutination reaction a comparison was made of 3 strains of P. pestis -- the avirulent 17 and EV, and the virulent KSh-13 (isolated in 1960 from the tick Rh. sanguineus) and strains of intestinal bacteria S. typhi murium 256 (isolated in 1959 from a suslik), S. suipestifer (reference strain), S. enteritidis 527 (isolated from a roller [bird] in 1960), Sh. sonnei 402 isolated from a rook [bird] in 1959), E. coli 0-111 (reference strain), and E. coli communis 479 (isolated from a Little Suslik in 1960). The results of the investigation are presented in table 3. All three strains of the plague microbe were mutually agglutinated in high titers and produced a cross reaction with E. coli communis 479 and S. typhi murium 256. Serological bonds between strains of P. pestis and other intestinal bacteria in these tests were expressed less clearly and had primarily a unilateral nature. For the serological interrelationships of the plague microbe and enterobacteria, judging by the material presented, it is characteristic that strains of enterobacteria are quite often agglutinated by plague serum, whereas cultures of the plague microbe react with intestinal sera considerably less often and in lower titers.

In the tests of the cross reactions of saturation, strains P. pestis 17 and E. coli communis 479, and P. pestis KSh-13 and E. coli communis 479 mutually lowered the titer of the heterologous sera; strain S. typhi murium 256 also partially extracted antibodies from the plague sera. The sera of E. coli 0-111 did not agglutinate P. pestis strain KSh-13, however, its titer following saturation by the stated strain was lowered by two times. Apparently, in the given case P. pestis strain KSh-13 possessed only an agglutinin binding ability. In a comparison of the strains of the plague microbe with Sh. sonnei 402 and S. suipestifer, an adsorbtion was not exposed.

Generally the tests of antibody adsorbtion supported the presence in several strains of the plague microbe and enterobacteria of common components, along with the specific antigens.

Conclusions

1. During an investigation of 346 strains of P. pestis with the agglutinating sera of bacteria of the intestinal group a positive result

was obtained in 31 cases (8.9%). Plague serum agglutinated 46 cultures of enterobacteria from various species out of 165 checked (27.8%).

2. In the tests of the cross reactions of agglutination and antibody adsorbtion, the presence of common antigenic components was supported in some of the strains of the plague microbe and enterobacteria.

3. The presence in strains of P. pestis of antigens which are inherent to bacteria of the intestinal group was not connected with other biological properties of the cultures, and the stated feature, judging by the data obtained, cannot be used for the intraspecies grouping of strains of the plague microbe.

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Table 1

Agglutination reaction of plague microbe strains with the antisera of intestinal bacteria

Agglutinating serum	Titer of serum	Number of positively reacting strains	Titer of positive reactions			
			1:25	1:50	1:100	1:200
S. paratyphi A	1:6400	2	-	-	2	-
S. typhi murium	1:25600	16	3	3	6	4
S. paratyphi C	1:6400	11	2	5	3	1
S. enteritidis	1:12800	12	3	3	2	4
E. coli 0-111	1:3200	5	3	2	-	-
E. coli 0-55	1:6400	4	2	1	1	-
E. coli 0-26	1:3200	1	2	-	-	

Note: With each of the stated sera, 345 strains of the plague microbe were tested.

Table 2

Agglutination reaction of bacteria of the intestinal group with plague serum

Name of bacteria	Number of strains checked	Of these, positive result	Titer of positive reactions					
			1:50	1:100	1:200	1:400	1:800	1:1600
S. paratyphi A	4	-	-	-	-	-	-	-
S. paratyphi B	8	5	1	2	-	-	1	1
S. typhi murium	21	13	1	1	2	2	5	2
S. suipestifer	3	1	-	-	1	-	-	-
S. typhi abdominalis	2	1	-	-	-	1	-	-
S. enteritidis	3	1	-	-	-	-	1	-
Shigella	7	2	1	-	-	-	1	-
Intermediate variants	36	10	2	2	1	2	3	-
E. coli communis	81	13	1	4	1	2	4	1

Table 3

Cross agglutination reaction between strains of the plague microb and enterobacteria

Strain / Serum	P.pestis 17 vac.	P.pestis EV	P.pestis KSh-13	S.typhi murium 256	S.suipestifer	S.enteritidis	Sh. sonnei 402	E.coli 0-111	E.coli communis 479
P. pestis 17 vac.	1:1280	1:1280	1:640	1:160	1:80	1:80	1:80	-	1:320
P. pestis EV	1:1280	1:1280	1:640	1:80	1:20	-	-	-	1:160
P. pestis KSh-13	1:1280	1:2560	1:640	1:80	-	-	1:20	-	1:160
S. typhi murium 256	1:320	1:320	1:40	1:5120	-	1:320	1:40	-	1:20
S. suipestifer	1:160	1:40	1:20	1:40	1:1280	1:40	1:10	-	-
S. enteritidis	1:20	1:160	-	1:640	1:40	1:1280	-	-	-
Sh. sonnei 402	-	1:40	-	1:20	-	-	1:1280	-	-
E. coli 0-111	1:20	1:80	1:20	-	-	-	-	1:640	1:40
E. coli communis 479	1:320	1:640	1:320	-	-	-	-	1:40	1:5120